OPTIMIZATION OF GLUCOSE PRODUCTION FROM ENZYMATIC HYDROLYSIS OF RICE STRAW USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Production cost of cellulase enzyme is very expensive. A research to reduce the cost by optimization of pH, temperature and agitation rate on production of glucose from enzymatic hydrolysis of rice straw by cellulose from *Aspergillus niger* using Response Surface Methodology (RSM) was successfully done. At the first stage of experiment, one factor at a time was employed to screen the best range for pH, temperature and agitation rate. All of the parameters ranges obtained were used in RSM. Response Surface Methodology in Design Expert version 6.0.8 software was used with Central Composite Design (CCD) mode. Seventeen sets of experiments with different parameters values were suggested by the software. The predicted optimum values for pH, temperature, agitation rate and concentration of glucose were 4.23, 43^{o}C, 177 rpm and 10.146 g/L respectively. One set of experiment was run using the optimized parameter and as a result, 9.9835 g/L concentration of glucose was recorded. Before optimization, concentration of glucose was only 5.5622 g/L and the concentration of glucose was increased by 44% after optimization. The optimization also reduces the energy consumption as the temperature was reduced from 45^{o}C to 43^{o}C and agitation rate reduced from 180 to 177 rpm. As conclusion, this research is successful to increase the concentration of glucose production, reduce the energy consumption and also be able to reduce the cost of production.

ABSTRAK

Kos penghasilan enzim sellulase sangat mahal. .Satu kajian untuk merendahkan kos penggunaan enzim dengan mengoptimumkan pH, suhu dan kadar adukan terhadap proses penghasilan glukosa melalui penggunaan enzim sellulase daripada *Aspergillus niger* terhadap jerami padi dengan menggunakan Kaedah Permukaan Tindak balas (RSM) telah berjaya dilakukan. Di awal peringkat eksperimen, kaedah satu faktor pada satu masa telah digunakan untuk menyaring julat pH, suhu dan kadar adukan yang terbaik. Kesemua julat parameter yang diperolehi, digunakan dalam Kaedah Permukaan Tindak balas (RSM). Kaedah Permukaan Tindak balas (RSM) dalam perisian Design Expert versi 6.0.8 telah digunakan dengan mod Rekabentuk Komposit Berpusat (CCD). Tujuh belas set eksperimen berlainan nilai parameter telah dicadangkan oleh perisian ini. Nilai optimum yang diramalkan untuk pH, suhu, kadar adukan dan kepekatan penghasilan glukosa masing-masing 4.23, 43^{o}C, 177 rpm dan 10.1460 g/L. Satu set eksperimen telah dijalankan bagi menguji parameter yang telah dioptimumkan dan sebagai keputusannya, 9.9835 g/L kepekatan glukosa telah direkodkan. Sebelum pengoptimuman, kepekatan glukosa hanyalah 5.5622 g/L dan penghasilan glukosa meningkat 44% selepas pengoptimuman. Pengoptimuman juga menurunkan penggunaan tenaga seperti suhu yang telah direndahkan dari 45^{o}C kepada 43^{o}C dan kadar adukan dikurangkan dari 180 rpm kepada 177 rpm. Sebagai konklusinya, kajian ini telah berjaya meningkatkan penghasilan glukosa daripada jerami padi, mengurangkan penggunaan tenaga dan juga mampu menurunkan kos penghasilan.

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LIST OF SYMBOLS/ABBREVIATIONS

Adj	-	Adjusted
ANOVA	-	Analysis of variance
CCD	-	Central composite design
cm	-	Centimeter
Corr	-	Correlation
DH	-	Degree of hydrolysis
dH_2O	-	Dilution of water
DNS	-	Dinitrosalicylic
GMC	-	Generic model control
g	-	Gram
g/L	-	Gram per liter
h	-	Hour
L	-	Liter
M	-	Molar
mg	-	Milligram
mg/ml	-	Miligram per milliliter
min	-	Minutes
ml	-	Mililiter
NaOH	-	Sodium hydroxide
nm	-	Nanometer
^oC	-	Degree Celcius
OD_{540}	-	Optical density at 540nm
OFAT	-	One factor at a time
Pred	-	Predicted
Prob	-	Probability
R^2	-	Coefficient of design
Rpm	-	Rotation per minutes

RSM	-	Response surface methodology
SEM	-	Scanning electron microscopy
SSF	-	Simultaneous sacharification and fermentation
T	-	Temperature
%	-	Percentage

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Lignocellulosic biomass is consisting of cellulose, hemicellulose and lignin. Biomass comes in many different types, there are wood residues, municipal paper waste, agricultural residues including corn stover and sugarcane bagasse, rice straw and dedicated energy crops. Many of the crops can provide high energy biomass which may be harvested multiple times each year (Sun *et al.*, 2008).

Biomass is a carbon source of energy. It is comes from dead plants, which means that the combustion of ethanol produced from lignocelluloses will produce no net carbon dioxide in the earth's atmosphere. Biomass is also readily available and the fermentation of lignocelluloses provides an attractive way to dispose of many industrial and agricultural waste products (Jin and Chen, 2006).

One of the most abundant lignocellulosic biomass is rice straw. Malaysia is one of the agricultural country and rice straw is the important agricultural corps in Malaysia with a production of 2 362 000 metric ton per year (Sarote and Jowaman, 2005). It was used as feedstock for paper industry, animal feed and organic fertilizer. In order to develop its new uses, the conversion of rice straw into glucose has been studied for fermentation process to produce ethanol.

Rice straw is difficult to convert into the fermentable sugar because the strong crystalline structure of cellulose in rice straw and the presence of the complex structure of lignin and hemicellulose with cellulose, which together limit the accessibility of rice straw to hydrolytic enzymes. Therefore, various pretreatments of rice straw have been developed to remove lignin and hemicelluloses, reduce cellulose crystallinity and increase the porosity thus increases its enzymic hydrolysis. There has been many pretreatment of lignocellulosic material such as acid treatment, alkaline treatment and oxidative delignification ozone (Ma *et al*., 2009).

Enzymatic hydrolysis provides a method to convert cellulose to glucose at high yields without sugar product degradation. Enzymatic hydrolysis of cellulose proceeds in several steps to break glycosidic bonds by the use of cellulase enzymes. Factors effecting hydrolysis of cellulose include type of substrate, cellulase loading and reaction conditions such as temperature, pH and end-product inhibitors. Cellulases are synthesized by fungi, bacteria and plants with most research focused on fungal and bacterial cellulases produced both aerobically and anaerobically. The aerobic mesophilic fungus, *Trichoderma reesei* QM 6a and its mutants have been the most intensely studied sources of cellulases. Cellulase is not a single enzyme but is made up of a family of at least three groups of enzymes: *1,4- β-D-glucan glucanohydrolases* (endoglucanases), 1,*4- β-D-glucan cellobiohydrolases* and *1,4- β-D-glucanglucohydrolases* (exoglucanases) and *β-D-glucoside glucohydrolases* (β-glucosidases) (Silverstein, 2004).

One of the examples from previous research about production of glucose by using enzymatic hydrolysis method is studied by Kunamneni and Singh (2005). This research studied the optimization of enzymatic hydrolysis of maize starch for higher glucose production. Crude amylases were prepared from *Bacillus subtilis* ATCC 23350 and *Thermomyces lanuginosus* ATCC 58160 under solid state fermentation. The effect of various process variables was studied for maximum conversion efficiency of maize starch to glucose using crude amylase preparations. Doses of pre-cooking-amylase, post-cooking-amylase, glucoamylase and saccharification temperature were found to produce maximum conversion efficiency and these were selected for optimization.

In statistics, Response Surface Methodology (RSM) explores the relationships between several explanatory variables and one or more response variables. The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response (Jones, 1996). As an example, Response Surface Methodology (RSM) has been extensively applied in optimization of enzymatic hydrolysis of *Cistus ladanifer* and *Cytisus striatus* for bioethanol production (Ferreira *et al.*, 2009). This reseach studied the optimization of enzymatic hydrolysis using the response surface methodology allowed a study on the influence of the variables (pH, temperature, cellulases concentration, polymer (PEG) concentration and incubation time) and variability due to the type of substrate (*C. ladanifer* and *C. striatus*) used. From the obtained results it can be concluded that the enzymatic hydrolysis was clearly enhanced by temperature, cellulase concentration and incubation time (Ferreira *et al.*, 2009).

1.2 Problem Statement

As crop residue after harvesting time, normally rice straw is discarded as a waste by farmers in Malaysia. This is cause the environmental issue. This research is one of steps to change the waste of rice straw to be valuable thing. Rice straw can be converted to reducing sugars which can be fermented to target product such as ethanol, lactic acid and single cell protein by suitable microorganism.

1.3 Objectives

As a huge potential in producing sugar from biomass, this research is carried out with the objective of optimization of glucose production from rice straw by enzymatic hydrolysis using Response Surface Methodology (RSM).

1.4 Scopes of Research

In order to achieve the above objective, the following scopes have been identified:

i. To study the effect of pH on production of glucose in enzymatic hydrolysis of rice straw.
ii. To study the effect of temperature on production of glucose in enzymatic hydrolysis of rice straw.
iii. To study the effect of agitation rate of shaking on production of glucose in enzymatic hydrolysis of rice straw.
iv. To optimize the pH, temperature and agitation rate on production of glucose using Response Surface Methodology (RSM).

1.5 Rational and Significant

One of the most abundant lignocellulosic biomass is rice straw. Malaysia is one of the agricultural country and rice straw is the important agricultural corps in Malaysia with a production of 2 362 000 metric ton per year (Sarote and Jowaman, 2005).

Rice straw is discarded as waste in Malaysia. This is causing environmental issue as the farmers usually just burn out the rice straw. This research is done as one of the steps to change the waste of rice straw into a valuable thing. The concept of changing waste to wealth is applied in this research.

In this study rice straw has been chosen to be the raw material of producing glucose in this research due to its abundant and low cost rather than using other source as well as it also clean, nontoxic and renewable.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Biorenewable resources are usually classified as either wastes or dedicated as energy crops. Categories of waste materials that qualify as biorenewable resources include agricultural residues, yard waste, municipal solid waste, food processing waste and manure. Agricultural residues such as rice straw, corn stover, rice hulls, wheat straw, cotton stalks and bagasse are the portion of the crop discarded after harvest (Sun *et al.*, 2008).

The straw has traditionally been removed from the field by the practice of open-field burning. This practice clears the field for new plantings and cleans the soil of disease-causing agents. Recently, the impact of open-field burning of rice straw on air quality has led to legislation which will in the future strictly control this practice. In the search for viable alternatives, the rice growers are considering straw as a source of liquid fuels and energy. The carbohydrate portion of the straw, 60% by weight, is being considered as a feedstock for fermentation, in a process that requires both a chemical pretreatment and enzymatic conversion of cell wall polysaccharides to monosaccharides (Yu *et al.*, 1996). Table 2.1 presents the potential supply of crop residues in some selected Southeast Asian countries during 1980/1981(Sarote and Jowaman, 2005).

Table 2.1: Potential supply of crops residues in some selected Southeast Asian countries during 1980/1981

	Indonesia	Malaysia	Thailand	Total
	1 X 10^3 metric ton			
Rice straw	36 300	2 362	20 900	81 881
Maize stover	3 991	8	3 700	11 415
Sweet potato vine	624	11	104	1 789
Cassava leaves	1 098	29	1 432	3 015
Banana stem and leaves	3 423	825	4 446	12 639
Banana fruit wastes	467	112	606	1 723
Pineapple wastes	186	145	1 260	2 676
Sugar-cane tops and leaves	5 268	255	5 580	18 408

Data from Table 2.1 indicate that the main food crops of the region are rice, sugar-cane, cassava, maize and other plantation crops such as banana stem and pineapple wastes. Data shows that Malaysia has the higher potential supply of crop residues for producing of rice straw. Bioconversion of rice straw for bioproduction of ethanol is flourishing as a result of increasing environmental pressure and decreasing fossil-fuel energy supply (Jin and Chen, 2006).

2.2 Rice Straw Composition

Most of the carbohydrate content of plants is structural polysaccharides that provide strength, shape and support for the plant. Lignocelluloses are complex structural material in the cell wall: cellulose, hemicelluloses and lignin are the three main components of lignocellulosic materials with other minor components being ash, protein and extractives (Silverstein, 2004). The distribution of cellulose, hemicelluloses and lignin in a typical plant cell wall are shown below in Figure 2.1.

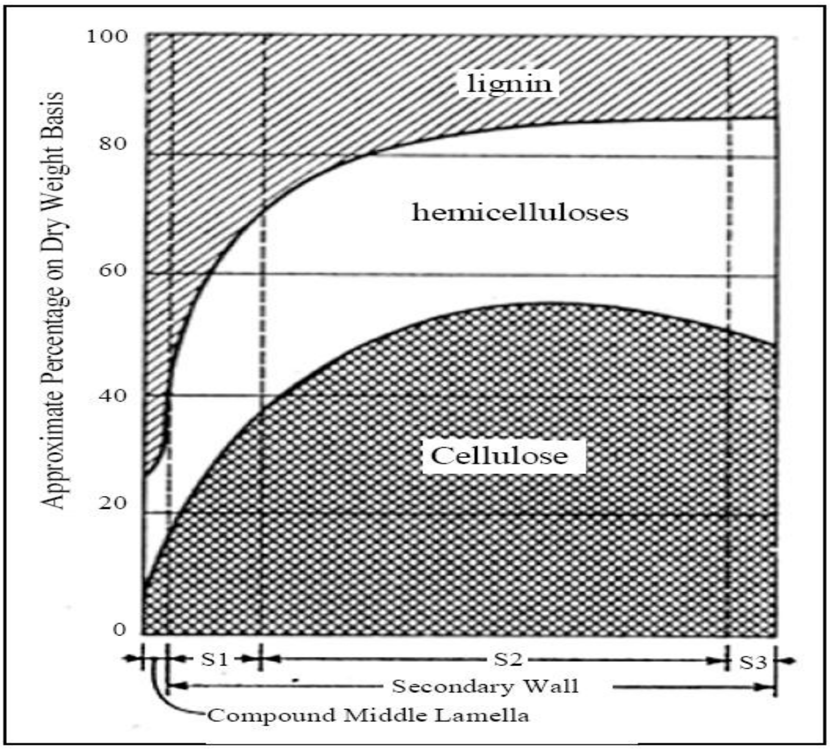

Figure 2.1: Distribution of cellulose, hemicelluloses and lignin in a typical plant cell wall

2.2.1 Lignin

Lignins are the most abundant aromatic plant component in terrestrial ecosystems and represent a significant part of plant litter input (approximately 20%) into soils (Crawford, 1981). In higher plants, lignins are chemically connected to cellulose and hemicellulose in the cellulosic fiber walls, providing strength and rigidity to the plant structures as well as resistance to the biodegradation of carbohydrates (i.e., enzymatic hydrolysis) and to environmental stresses (Brown, 1961).

Lignins are synthesized from L-phenylalanine and cinnamic acids via various metabolic ways to form lignin precursors such as sinapyl and coniferyl alcohols (Higuchi, 1971). The lignin structure consists of aromatic rings with side chains and alkyl alcohol and methoxy groups linked by various strong covalent bonds (alkyl-aryl ether and alkoxyalkane). Lignins are synthesized by oxidative copolymerization of three p-hydroxycinnamyl alcohols (p-coumaryl, coniferyl and sinapyl) which contribute in varying proportions to the macromolecular structure depending upon the morphological parts of plants (Adler, 1977).

Lignin plays a crucial part in conducting water in plant stems. The polysaccharide components of plant cell walls are highly hydrophilic and thus permeable to water, whereas lignin is more hydrophobic. The cross linking of polysaccharides by lignin is an obstacle for water absorption to the cell wall. Thus, lignin makes it possible for the plant's vascular tissue to conduct water efficiently (Silverstein, 2004).

Various chemical or biological treatments have been used to remove or modify lignin and to cleave lignin-matrix cross-links in cell walls, but the specificity of these treatments was poor, making it difficult to attribute changes in degradability to specific changes in cell wall properties. Complex structure of lignin and hemicelluloses with cellulose, which together limit the accessibility of rice straw to hydrolytic enzymes (Thevenot *et al.*, 2010). Therefore, various pretreatments of rice straw have been developed to remove lignin, hemicelluloses, reduce cellulose crystallinity and increase the porosity thus increases its enzymic hydrolysis. There has been many pretreatment of lignocellulosic material such as acid treatment, alkaline treatment and oxidative delignification ozone. The structure of a small section of a lignin polymer is shown below in Figure 2.2 (Silverstein, 2004).

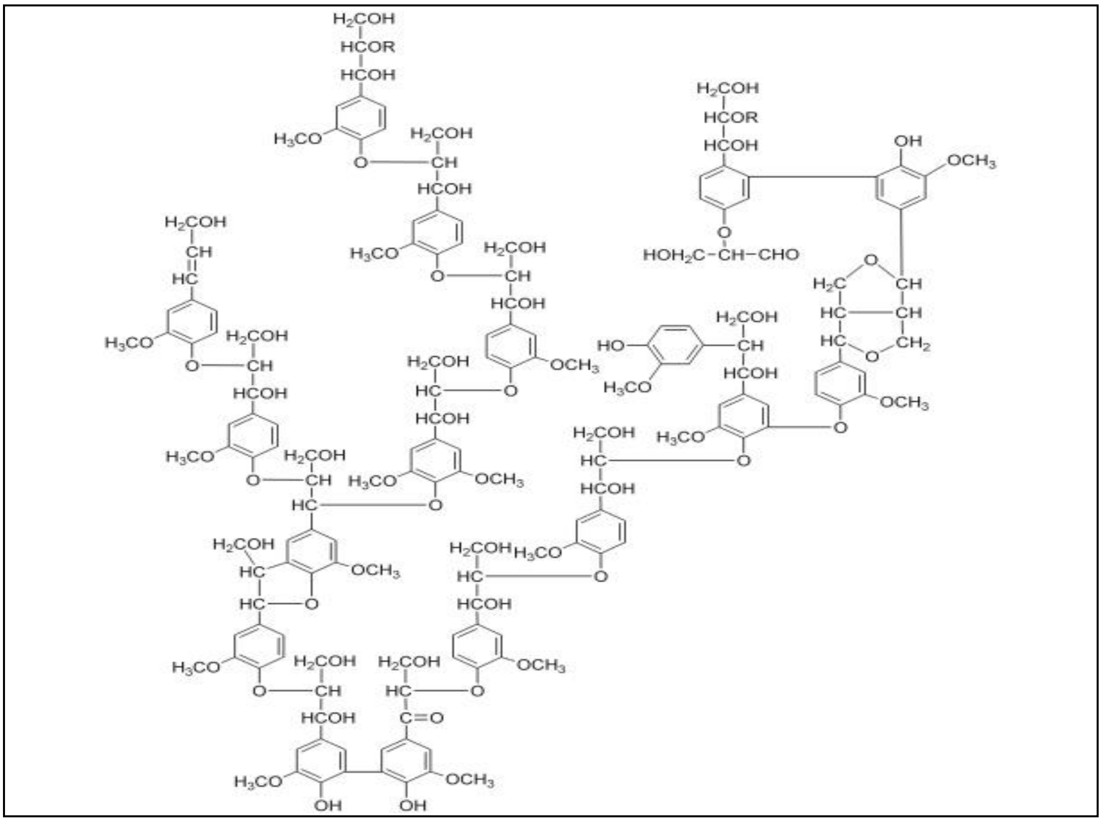

Figure 2.2: Structure of a section of a lignin polymer

2.2.2 Hemicellulose

Hemicelluloses are complex, highly branched polysaccharides that occur in association with cellulose in the cell walls. The monomers that comprise hemicellulose are hexoses (glucose, galactose and mannose) and pentoses (arabinose and xylose). Hemicellulose can be classified into three groups, namely, xylans, mannans and 1,3 galactans based on the polymer backbone that is very often homopolymeric with β-1,4 linkages (Brigham *et al*., 1996).

In softwoods, the primary hemicellulose components are galactoglucomannans and arabinoglucuronoxylan while the principal hemicelluloses in hardwoods are glucomannans and methylglucoronoxylans (Brigham *et al*., 1996). Xylan is the most important in terms of the percentage of total hemicellulose found in biomass. Galactoglucomannan consists of β- 1,4-linked mannose and glucose units in a ratio of 3:1 to which O-acetyl groups and α-1,6- linked galactose side groups are attached (Puls and Schuseil, 1993).

Hemicelluloses are one of the most abundant natural polysaccharides and comprise over 30% of the dry mater of rice straw. They unlike cellulose which is a unique molecule differing only in degree of polymerization and crystalline are inhomogeneous fractions and classically defined as the alkali soluble material after removal of the pectic substances (Sun *et al.*, 2000). Figure 2.3 shows the structure of hemicelluloses.

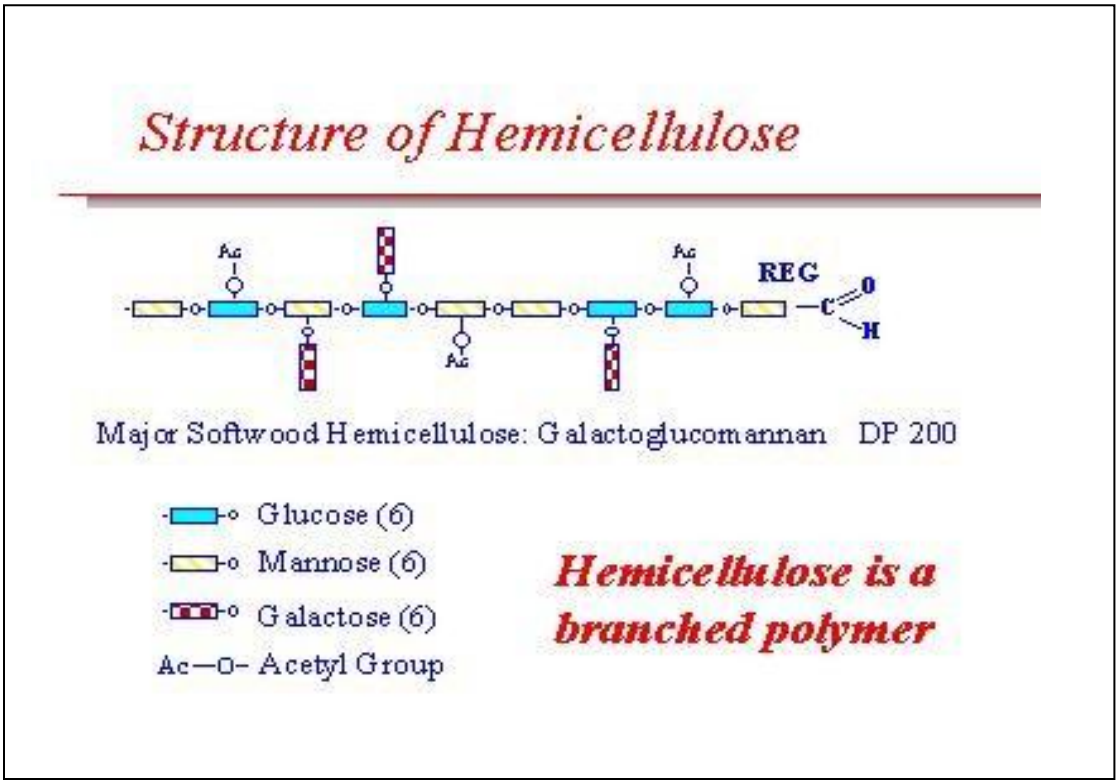

Figure 2.3: Structure of hemicellulose

2.2.3 Cellulose

Cellulose is a linear polymer of anhydro D-glucose units connected by β-1,4 glycosidic bonds as shown below in Figure 2.4. Native cellulose exists in the form of microfibrils which are paracrystalline assemblies of several dozen (1→ 4) β-D glucan chains held together by intermolecular hydrogen bonds. Intramolecular hydrogen bonds also form between two glucose units in the same chain (Carpita and McCann, 2000).

The combined bonding energies of the intermolecular and intramolecular hydrogen bonds increases the rigidity of cellulose and forms the crystalline structure that makes it highly insoluble and recalcitrant to most organic solvents. The cellulose microfibrils are imbedded in a matrix of noncellulosic polysaccharides, mainly hemicellulose and pectic substances (Silverstein, 2004), which complicates hydrolysis of cellulose to glucose even further. The cellulose in lignocellulosic biomass feedstocks provides the main source of glucose used during ethanol fermentation. Figure 2.4 shows the structure of cellulose.

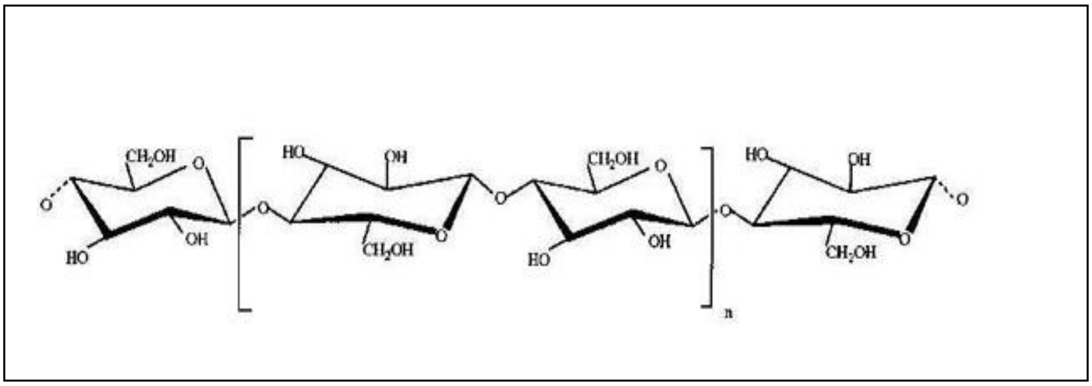

Figure 2.4: The structure of cellulose